(19) Europäisches Patentamt
European Patent Offic
Office eur péen des brevets

(11) Publication number: 0 171 084 B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: 05.06.91

(51) Int. Cl.5: A61K 31/19, A61K 9 10, A61K 47:00

(21) Application number: 85110009.9

(22) Date of filing: 08.08.85

The file contains technical information submitted after the application was filed and not included in this specification

(54) **Pharmaceutical oil-in-water type micro-emulsion.**

(30) Priority: 10.08.84 JP 166612/84

(43) Date of publication of application: 12.02.86 Bulletin 86/07

(45) Publication of the grant of the patent: 05.06.91 Bulletin 91/23

(84) Designated Contracting States: BE CH DE FR GB IT LI NL SE

(56) References cited:
EP-A- 0 129 435
DE-A- 1 805 003
GB-A- 2 046 094

PATENT ABSTRACTS OF JAPAN, vol. 8,no.122, (C-227)(1559),June 8,1984 & JP-A-59 33 244

(73) Proprietor: LEDERLE (JAPAN) LTD.
Hattori Bldg., 5th Floor 10-3 Kyobashi
1-chome
Chuo-ku Tokyo(JP)

Proprietor: MORISHITA PHARMACEUTICAL CO. LTD.
29 Doshomachi 4-chome Higashi-ku
Osaka(JP)

(72) Inventor: Mizushima, Yutaka
25-20, Daita 4-chome
Setagaya-ku Tokyo(JP)
Inventor: Fujii, Mitsuharu
1823-1, Ohshinohara Yasu-cho
Yasu-gun Shiga-ken(JP)
Inventor: Takei, Hiroshi
747-7, Komejima Showa-machi
Kitakatsushika-gun Saitama-ken(JP)

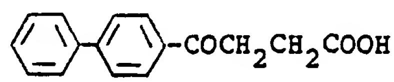
(74) Representative: Kraus, Walter, Dr. et al
Patentanwälte Kraus, Weisert & Partner
Thomas-Wimmer-Ring 15
W-8000 München 22(DE)

EP 0 171 084 B1

Description

This invention relates to a novel pharmaceutical composition in form of an oil-in-water type micro-emulsion as defined below which has excellent anti-inflammatory, analgesic and antipyretic activities and can also be administered parenterally, a process for preparation of the composition and this pharmaceutical composition for use in the treatment of inflammations, pain and fever.

4-Biphenylylacetic acid is a known compound having strong anti-inflammatory, analgesic and antipyretic activities (see U. S. Patent 3,784,704). It is known, however, that oral or parenteral administration of 4-biphenylylacetic acid may sometimes be accompanied by ulceration or bleeding of the digestive organs. Hence, in spite of its excellent antiinflammatory, analgesic and antipyretic activities, it has not yet been used in clinical therapy as a practical drug. In order to reduce the side-effects of 4-biphenylylacetic acid while retaining its excellent pharmacological activities, 4-(4-biphenyl)-4-oxobutyric acid (common name: fenbufen; tradename NAPANOL®, CINOPAL®) of the following formula



was developed on the basis of the pro-drug theory (see Arzneimittel Forschung, 30 (1), 693-746, 1980), and this compound has been widely used clinically as an orally administrable anti-inflammatory, analgesic and antipyretic agents.

Fenbufen exhibits its anti-inflammatory, analgesic and antipyretic effects after it is converted to 4-biphenylylacetic acid within the body. Metabolization to the active substance, 4-biphenylylacetic acid, requires time and its effects appear somewhat slowly.

The side-effects of fenbufen on the digestive organs are considerably reduced as compared with conventional non-steroidal anti-inflammatory agents. But it is better to use with caution when applied to patients with a history of peptic ulcer or administered in large amounts.

Recently, the targeting therapy in which a drug is administered as a dissolved form in lipid particles of a lipid emulsion has been proposed and aroused much interest. This therapy utilizes the property of lipid particles, like liposome, to be taken into the reticulo-endothelial system or inflamed cells. When a lipid-soluble drug dissolved in such lipid particles is administered, the lipid particles act as a drug carrier to carry the drug selectively to a specific site where the effect of the drug is exhibited concentratingly.

As such drugs, emulsions obtained by incorporating dexamethasone palmitate, ibuprofen, flufenamic acid, indomethacine ester, prostaglandin E_1, and ketoprofen or its alkyl esters in lipid particles and emulsifying the lipid particles in water have been proposed [see, for example, A. Yanagawa, Japanese Journal of Inflammation, vol. 2, No. 3, Summer (1982), pages 251-257, Japanese Laid-Open Patent Publications Nos. 16818/1982, 59912/1983, 201712/1983, 222014/1983, and 13720/1984]. Among them, dexamethasone palmitate and prostaglandin E_1 are lipid soluble and have successfully been formed into stable lipid emulsions. They are being clinically tested for administration to humans. The other drugs do not have sufficient solubility in oils or fats such as soybean oil. Then lipid emulsions having low concentrations of drug for animal tests can be prepared from these drugs, but no stable lipid emulsion having a sufficient concentration of the active compound to produce a satisfactory clinical effect has been obtained from them. Hence, the work to develop such lipid emulsions has been suspended.

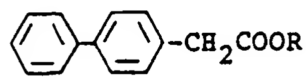
GB-A-2 046 094 teaches a solution of anti-inflammatory acrylacetic and arylpropionic acid derivatives which is stabilized with the addition of phospholipids and which is a parenterally administrable solution. This citation merely describes the anti-inflammatory acrylacetic and arylpropionic acid derivatives as drugs and does not provide any teaching or suggestion for 4-biphenylylacetic acid and how to prepare a lipid emulsion.

Thus, the prior art has not given sufficient results in the preparation of lipid emulsions although an excellent therapeutic effect will be expected from the administration of drugs having anti-inflammatory, analgesic and antipyretic activities as lipid emulsions. It has been strongly desired therefore to develop a drug composition which can rapidly produce effects without an impairment in anti-inflammatory, analgesic and antipyretic activities with a minimum of the aforesaid side effects.

It is an object of this invention to provide a pharmaceutical oil-in-water type micro-emulsion comprising fine particles of an oil or fat dispersing said fine particles in said aqueous medium.

Another object of this invention is to provide a process for preparing the aforesaid pharmaceutical oil-in-water type micro-emulsion.

This invention relates to a pharmaceutical oil-in-water type micro-emulsion comprising fine particles having a mean particle diameter of from 0.1 to 1.0 μm of a vegetable oil or a triglyceride of a medium-chain fatty acid having 8 to 12 carbon atoms containing 0.01 to 10% (w/v) of a 4-biphenylylacetic acid ester of the formula



(I)

wherein R represents an alkyl group having 1 to 18 carbon atoms,
an aqueous medium, and 0.05 to 25% (w/v) of a physiologically acceptable phospholipid for dispersing said fine particles in said aqueous medium.

This invention relates also to a process for producing a pharmaceutical oil-in-water type micro-emulsion which comprises dissolving a predetermined amount of said 4-biphenylylacetic acid ester in said vegetable oil or triglyceride under heat, adding a predetermined amount of the phospholipid and as required other additives, stirring the mixture to form a uniform mixture, then adding water, treating the mixture with a dispersion homogenizer to prepare an oil-in-water type crude emulsion, and thereafter homogenizing the crude emulsion by a high-energy homogenizer.

The 4-biphenylylacetic acid ester of formula I used in this invention has a strong affinity for, and are well miscible with, oils or fats such as soybean oil, and forms stable micro-emulsion containing the 4-biphenylylacetic acid ester in clinically effective concentrations. When the resulting micro-emulsion containing the 4-biphenylylacetic acid ester is administered to a mammal, it exhibits much stronger anti-inflammatory, analgesic and antipyretic activities than when the 4-biphenylylacetic acid itself is administered in solution or suspension forms. Moreover, its side effects such as disturbance in the digestive organs are drastically reduced, and its high activities appear rapidly and last over an extended period of time.

Accordingly, since the 4-biphenylylacetic acid ester of formula (I) has better compatibility with oils or fats, the micro-emulsion of this invention has greater stability than the lipid emulsion of 4-biphenylylacetic acid itself. The 4-biphenylylacetic acid ester of formula (I) dissolved in lipid particles is distributed efficiently to a site of inflammation and hydrolyzed _in situ_ by the action of esterase to 4-biphenylylacetic acid, the active substance, and consequently exhibits its excellent effects.

The emulsion of the present invention is a systemically administrable micro-emulsion which has enabled 4-biphenylylacetic acid to be used in clinical therapy for the first time. It is a valuable preparation which greatly contributes to the medical field, especially in the therapy of inflammation, pain and fever.

Figure 1 is a graph showing the results of measuring the analgesic activity of the micro-emulsion of the present invention;
Figure 2 is a graph showing the results of measuring the distribution of the micro-emulsion of the invention to the tissues; and
Figures 3 to 7 are graphs showing the results of clinical tests of the micro-emulsion of this invention.

Of the 4-biphenylylacetic acid esters of formula (I), those having high lipophilicity are preferred in this invention.

In formula (I) representing the 4-biphenylylacetic acid ester, R represents
alkyl groups having 1 to 18 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isoamyl, n-pentyl, n-hexyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl and octadecyl. Lower alkyl groups such as methyl and ethyl are preferred, and the ethyl group is most preferred.

The term "lower", used in the present specification, means that a group or compound qualified by this term has not more than 6, preferably not more than 4, carbon atoms.

It is presumed that the 4-biphenylylacetic acid ester exhibits its pharmacological effect when converted to 4-biphenylylacetic acid _in vivo_ by the enzymatic action of esterase.

The pharmaceutical micro-emulsion of this invention is prepared by introducing the compound of formula (I) into particles of an oil or fat used in the preparation of ordinary lipid emulsions. For example it can be easily prepared by dissolving the compound of formula (I) in fine particles of the oil or fat, and dispersing the fine particles in water using an emulsifier to form an oil-in-water emulsion.

The vegetable oil or triglyceride of a medium-chain fatty acid which can be used in preparing the pharmaceutical micro-emulsion of this invention includes any pharmaceutically acceptable oil and fat which are normally used. Specific examples of the vegetable oil include soybean oil, cottonseed oil, rapeseed oil and safflower oil, examples of the triglycerides of medium-chain fatty acids having 8 to 12 carbon atoms

include caprylic acid, capric acid and lauric acid, normally abbreviated as MCT. They may be used either singly or in combination. Among them, vegetable oils and Panacet®810 (MCT mixture, a product of Nippon Oils and Fats Co., Ltd.) are preferably used, and pharmaceutically acceptable soybean oil fitting the standards of medicines stipulated in Japanese Pharmacopoeia is most preferred. The amount of such an oil or fat is not strictly limited, and can be varied widely depending upon the type or amount of the pharmacologically effective compound of formula (I) and/or the other ingredients. Generally, it is 1 to 50 % (w/v), preferably 3 to 30 % (w/v), more preferably 5 to 20 % (w/v).

Unless otherwise stated, all percentages "% (w/v)" used to denote the contents or amounts used of the ingredients of the pharmacetucial micro-emulsion in the present specification and the appended claims mean parts by weight per 100 parts by volume of the final pharmaceutical micro-emulsion.

Examples of the physiologically acceptable phospholipids include yolk phospholipid, vegetable oil phospholipids such as soybean phospholipid, and phosphatidyl choline. These phospholipids can be used either singly or in combination. Preferably, the phospholipids used in this invention generally have an HLB of 6 to 15, preferably 10 to 14. Among the above phospholipids, yolk phospholipid and vegetable phospholipids such as soybean phospholipid are preferred. The vegetable oil phospholipids, particularly purified soybean phospholipid, are most suitable because they have a better emulsifying power and can form more uniform, finer and more stable phospholipid particles than the yolk phospholipid. Desirably, the vegetable oil phospholipids are purified to such an extent that the phosphatidyl choline content reaches at least 50 % by weight, preferably at least 80 % by weight. The soybean oil phospholipid so purified may have an iodine value of generally 30 to 50, preferably about 40.

The phospholipid is used in an amount of 0.05 to 25% (w/v), preferably 0.2 to 6% (w/v), more preferably 0.6 to 2.4 % (w/v). On the basis of the oil or fat, the suitable amount of the phospholipid is 6 to 24 parts by weight, especially 6 to 15 parts by weight, per 100 parts by weight of the oil or fat.

In the micro-emulsion of the present invention, a moderate amount of distilled water or deionized water may be used as the aqueous dispersion medium. If required, a small amount of a pharmaceutically acceptable water-miscible organic solvent such as ethanol may be incorporated.

As required, an isotonizing agent and other additives such as an emulsification aid and a stabilizer may further be incorporated in the micro-emulsion of the present ivnention.

Examples of the isotonizing agent include glycerol, sugar alcohols such as sorbitol and xylitol; monosaccharides such as glucose and fructose; disaccharides such as maltose; and amino acids such as L-alanine, L-valine and glycine. Of these, glycerol is especially suitable.

The isotonizing agent is added to adjust the osmotic pressure of the micro-emulsion to a value nearly equal to that of a body fluid. The amount of the isotonizing agent is such that its final concentration in the micro-emulsion is 0.1 to 0.5 mole/liter, preferably 0.25 to 0.35 mole/liter. More specifically, it can be incorporated usually in the following proportions depending upon the type of the isotonizing agent.

	Proportions, % (w/v)		
Isotonizing agent	General range	Preferred range	Most preferred range
Glycerol	2-4	2-3	about 2.5
Sugar alcohol	2-6	2.5-5	3-4
Monosaccharide	4-6	4.5-5.5	about 5
Disaccharide	8-12	9-11	about 10
Amino acid	3-5	3-4	about 3.5

Examples of the emulsifying aid that can be incorporated include fatty acids having 10 to 20 carbon atoms (such as stearic acid, palmitic acid, linoleic acid and linolenic acid) and salts thereof (such as sodium and potassium salts), phosphatidyl ethanolamine, phosphatidyl serine and stearylamine. It may be used generally in an amount of up to 0.4 % (w/v), preferably 0.01 to 0.2 % (w/v). In particular, the fatty acid or its salt can be advantageously used in an amount of 0.01 to 0.1 % (w/v), and phosphatidyl ethanolamine, phosphatidyl serine and stearylamine may be advantageously used in an amount of 0.05 to 0.3 % (w/v), especially 0.1 to 0.2 % (w/v).

Cholesterol or tocopherol, for example, may be used as a stabilizer. Conveniently, cholesterol may be

used generally in an amount of up to 1.2 % (w/v), preferably 0.2 to 0.4 % (w/v), and tocopherol may conveniently be used in an amount of up to 2.5 % (w/v), preferably 0.2 to 0.8 % (w/v).

Albumin, its fatty acid amide derivatives, and polysaccharides or their fatty acid ester derivatives may also be used as the stabilizer. From the standpoint of antigenicity, albumin is desirably one derived from a human when preparing a pharmaceutical micro-emulsion for humans. The fatty acid amide derivatives thereof may, for example, be compounds obtained by amidating 5 to 40 % of the entire amino groups present in albumin with fatty acids having 14 to 18 carbon atoms (such as palmitic acid and stearic acid). Examples of the polysaccharides include dextran, pullulan and hydroxyethyl starch. The fatty acid ester derivatives of these polysaccharides may be compounds obtained by, for example, esterifying 5 to 40 % of the entire hydroxyl groups present in the polysaccharides with fatty acids having 14 to 18 carbon atoms such as palmitic acid and stearic acid. The stabilizer may be added generally in an amount of 0.02 to 5 % (w/v), preferably 0.2 to 2.5 % (w/v).

The micro-emulsion of this invention may be prepared by using emulsifying methods known per se. Ordinary homogenizers may be used as an emulsifying machine. To prepare a stable lipid micro-emulsion, it is convenient to use two types of homogenizers. Specifically, the micro-emulsion of this invention may be prepared by dissolving an effective amount of the 4-biphenylylacetic acid ester in the oil or fat such as pharmaceutically acceptable soybean oil optionally under heat, adding a predetermined amount of an phospholipid such as refined soybean phospholipid and as required an isotonizing agent and other additives such as an emulsification aid or a stabilizer, stirring the mixture under heat to make a uniform mixture, adding water, and treating the mixture in a homogenizer to prepare a crude emulsion of the oil-in-water type, and thereafter, homogenizing the crude emulsion by a pressurized homogenizer such as Gaulin high-energy homogenizer. The stabilizer and the isotonizing agent may be added to the resulting micro-emulsion.

Desirably, the above emulsifying operation is carried out generally until the dispersed oil or fat particles in the resulting emulsion have a mean particle diameter of not more than 1 μm, preferably not more than 0.3 μm, more preferably 0.1 to 0.15 μm.

The 4-biphenylylacetic acid ester of formula (I) as a pharmacologically active ingredient is conveniently used so that its concentration generally becomes 0.01 to 50 % (w/v), preferably 0.01 to 10 % (w/v), more preferably 1 to 5 % (w/v).

As required, the micro-emulsion of this invention so prepared may be lyophilized. The powder obtained by lyophilization can be converted back to the original micro-emulsion when it is dissolved in water. It should be understood that the term "micro-emulsion", as used in the present application, also denotes such a lyophilized form of the micro-emulsion.

Thus, according to one preferred embodiment of this invention, there is provided a pharmaceutical oil-in-water type micro-emulsion consisting essentially of 5 to 50 % (w/v) of fine particles of an oil or fat containing 0.01 to 10 % (w/v) of the 4-biphenylylacetic acid ester of formula (I), 0.05 to 25 % (w/v) of a physiologically acceptable phospholipid an isotonizing agent in an amount sufficient to isotonize the emulsion, a water.

According to a more preferred embodiment of this invention, there is provided a pharmaceutical oil-in-water micro-emulsion consisting essentially of 5 to 30 % (w/v) of fine particles of soybean oil having dissolved therein 1 to 5 % (w/v) of ethyl 4-biphenylylacetate, 0.5 to 2.5 % (w/v) of purified soybean oil phospholipid, 1 to 5 % (w/v) of glycerol, and the remainder being water.

The micro-emulsion of this invention containing the 4-biphenylylacetic acid ester has excellent transferability to a site of inflammation (incorporation in inflamed cells) when administered parenterally by injection, for example. As a result, the pharmacologically effective compound exhibits its pharmacological effect strongly and concentratedly at the the site of inflammation for an extended period of time. In addition, its side effects and toxicity on the digestive organs are very little. The micro-emulsion has excellent stability and is very useful as an anti-inflammatory, analgesic and antipyretic agent.

The excellent pharmacological effects, low toxicity and high stability of the micro-emulsion of this invention are demonstrated by the following experiments.

[A] Pharmacological tests

A-1: Effect of inhibiting carrageenin-induced paw edema

[Test 1]

(a) Experimental animals: Wistar strain male rats (body weight 160-220 g), 7 per group.

(b) Test drugs:

The emulsion containing methyl 4-biphenylylacetate or ethyl 4-biphenylylacetat in a concentration of 2 % (w/v), calculated as 4-biphenylylacetic acid prepared by Example 1 or 2 given hereinafter was diluted to 40, 80, and 160 times, with physiological saline, and each of the solutions was administered at a dose of 10 ml per kg of the animal. As a control drug, an aqueous solution of sodium 4-biphenylylacetate was used.

(c) Experimental procedure:

The volume of the left hind paw of each rat was measured with a plethysmometer (supplied by Ugo Basile Company). A 1% carrageenin solution as an inflammation inducer was injected subcutaneously into the left hind paw of each rat to induce paw edema. The volume of the paw of the rat was measured before the administration of carrageenin and every hour after the administration up to 6 hours. The test drug was used in three doses of 1.25 mg/kg, 2.5 mg/kg, and 5.0 mg/kg. Sodium 4-biphenylylacetate as a control drug was used in two doses of 2.5 mg/kg and 5.0 mg/kg. The test drugs were intravenously administered 2 hours after the injection of carrageenin. The edema inhibition rate were calculated by the following equation.

$$\text{Edema inhibition rate (\%)} = \frac{Ec - Et}{Ec} \times 100$$

Ec: the volume of edema of the control group to which only the solvent was administered at each time (average value)

Et: the volume of edema in the drug-administered group at each time (average value)

(d) Experimental Results:

The results are shown in Table 1.

The micro-emulsion of this invention containing methyl 4-biphenylylacetate showed a significant edema inhibitive effect at any of the doses from 1 hour after the administration of the drug. Its activity lasted until 4 hours after the administration of the drug (6 hours after carrageenin administration). Likewise, the micro-emulsion of this invention containing ethyl 4-biphenylylacetate showed a significant edema inhibitive effect at doses of 2.5 mg/kg and 5.0 mg/kg from 2 hours after the administration of the drug and its effect was long-lasting. On the other hand, sodium 4-biphenylylacetate as a control drug showed a less effect than that of the above micro-emulsion. On the basis of these experimental results, the 20% edema inhibitory effect (ED_{20}) at 2 hours after administration was calculated from the dose-response curve. It was found that the micro-emulsions of this invention containing methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate respectively showed about 6 and about 3 times as strong an effect as the control sodium 4-biphenylylacetate.

Table 1

Effects on Carrageenin-induced Paw Edema in Rats [Test 1]

Drug	Dose (mg/kg) a)	Number of animals	Edema inhibitory effect (inhibition rate, %) 2 hours b)	3 hours	4 hours	5 hours	6 hours	ED_{20} (mg/kg)
Micro-emulsion containing methyl 4-biphenylylacetate	1.25	7	1.8	c) 18.9*	21.7**	24.6**	18.4**	1.25
	2.5	7	1.8	18.9*	24.6**	24.6**	23.4**	
	5.0	7	3.6	28.0**	34.8**	41.5**	35.0**	
Micro-emulsion containing ethyl 4-biphenylylacetate	1.25	7	2.2	3.3	8.7	11.9	9.9	3.0
	2.5	7	2.2	4.9	19.1*	28.8**	18.4*	
	5.0	7	2.2	8.2	23.5**	30.6**	27.9**	
Sodium 4-biphenylylacetate	2.5	7	0	8.4	13.2	17.1	19.8	8.5
	5.0	7	0	9.5	15.1	17.4	20.8	

a): Calculated as 4-biphenylylacetic acid

b): Times after injection of carrageenin (the drug was administered 2 hours after the injection of carrageenin)

c): Statistically analyzed figures against the solvent control group. * p<0.05, ** p<0.001

[Test 2]

(a) Experimental animals:

SD-strain male rats (120 - 180 g)

(b) Test Drugs:

A micro-emulsion containing ethyl 4-biphenylylacetat prepared as in Exampl s 11, 12, 13 and 14 given hereinafter was administered at a dose of 5 ml per kg of the animals. Venolipid® (Morishita Pharmaceutical Co., Ltd.) was used as a vehicle control. As control drugs, a solution or suspension of ethyl 4-biphenylylacetate in physiological saline containing 0.5% Cremophor EL® (Sigma Chemical Company, a solubilizing agent), 0.2 % of polyethylene glycol 400 and 10% of dimethyl sulfoxide, a physiological saline solution of sodium 4-biphenylylacetate and a physiological saline solution of Aspirin®DL-lysine [Venopyrin®, Green Cross Co.] were used. As the vehicle control of the control drugs, the above solvents were used.

(c) Experimental procedure:

A 1% carrageenin solution in physiological saline was injected subcutaneously in an amount of 0.05 ml into the planter surface of the left hind paw of each rat via a 25 gauge needle fitted to a 0.25 ml syringe. One hour after carrageenin injection, the rats were randomized on the basis of the edema volume, and intravenous administration of the drugs and vehicles followed immediately. The volume of the left hind paw was measured by a plethysmometer (Ugo Basile) prior to the injection of the carrageenin solution and at 2, 3, 4, 5 and 6 hours after the carrageenin injection. As the volume of edema, an increase in the paw volume was taken.

The inhibition percents were calculated in accordance with the same calculation formula as in Test 1.

(d) Experimental results

The micro-emulsion of this invention in intravenous injection showed an excellent anti-inflammatory activity against the carrageenin-induced paw edema in a dose range of 1.25 to 10.0 mg/kg, calculated as 4-biphenylylacetic acid. The ED_{30} value was 4.2 mg/kg, calculated as 4-biphenylylacetic acid. In contrast, the physiological saline solution of ethyl or sodium 4-biphenylylacetate, which is different from the micro-emulsion of this invention, showed an anti-inflammatory effect in a dose range of 2.5 to 10.0 mg/kg, calculated as 4-biphenylylacetic acid, but its ED_{30} was 15.8 and 10.5 mg/kg.

It can be found that the micro-emulsion of this invention shows two to three times as strong an effect as the ordinary solution form.

Table 2. Effects on Carrageenin-induced Paw Edema in Rats [Test 2]

Test drugs	Dose a) (mg/kg)	No. of Animals	Inhibition (%): Time after Carrageenin Injection 2 h	3 h	4 h	5 h	6 h	b) ED_{30} (mg/kg)
Micro-emulsion of ethyl 4-biphenylyl-acetate	1.25	7	12.7*c)	17.5*	13.7	12.0	14.0	4.2
	2.5	7	25.5**	36.8**	39.2**	40.0**	31.9**	
	5.0	7	25.5**	31.6**	31.4**	36.0**	27.7*	
	10.0	7	30.9**	36.8**	39.2**	40.0**	31.9**	
Ethyl 4-biphenylyl-acetate	1.25	7	14.0	17.5	9.6	9.8	6.4	15.8
	2.5	7	19.3*	26.3**	17.3**	17.6*	12.8	
	5.0	7	17.5*	28.1**	23.1**	23.5**	19.1	
	10.0	7	22.8*	22.8*	26.9**	25.5*	27.7*	
Sodium 4-biphenylyl-acetate	1.25	7	6.6	0	8.5	5.2	6.1	10.5
	2.5	7	19,7	11.7	18.6**	20.7*	10.2	
	5.0	6	9.8	6.6	20.3**	19.0**	30.6**	
	10.0	7	23.0*	28.3*	35.6**	29.3**	30.6**	
Aspirin® DL·Lysine	50	7	24.2	26.7*	35.7*	29.6*	30.0*	81.6
	100	7	24.2*	26.7*	26.8	22.2	20.0	
	200	7	37.9**	43.3**	50.0**	42.6**	44.0**	

a) Doses of the micro-emulsion of ethyl 4-biphenylylacetate, ethyl 4-biphenylylacetate and sodium 4-biphenylylacetate are expressed as those of 4-biphenylylacetic acid, and doses of aspirin® DL-lysine as those of aspirin®.

b) Dose required to cause a 30% inhibition of edema volume against the vehicle control. This value was calculated from the mean % inhibition of swelling combined for measurement intervals indicated.

c) * $P<0.05$, ** $p<0.01$

A-2. Inhibitory activity of adjuvant-induced arthritis

[Test 1]

(a) Experimental animals:

CRJ-CD(SD)-strain female rats (5 to 7 weeks old), 6 per group

(b) Test drugs:

Micro-emulsions containing methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate in a concentration of 2 % (w/v) calculated as 4-biphenylylacetic acid (prepared as in Examples 1 and 2 below) were diluted with Venolipid® (Morishita Pharmaceutical Co., Ltd.) to 20, 40 and 80 times, and administered at a dose of 10 ml per kg of the animals. Venolipid was used as the vehicle control.

(c) Experimental procedure:

A suspension containing 0.6 mg of heated dead cells of Mycobacterium butyricum in liquid paraffin as an adjuvant was injected into the left hind paw of each rat. Each of the test drugs was administered into the tail vein for 5 days starting from the 15th to 19th day after the injection of the adjuvant. The volume of the hind paw was measured by a plethysmometer (supplied by Ugo Basile Company) at the specified times till the 25th day after the injection of the adjuvant. The edema inhibition rate was calculated in accordance with the same calculation formula as in [Test 1] of A-1.

(d) Experimental results:

The results are shown in Table 3 below. The edema at the left hind paw injected with the adjuvant was significantly inhibited by the intravenous administration of the micro-emulsions containing methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate respectively in each of the doses, and the inhibitory action showed dose-dependence.

Table 3

Effects on Adjuvant-induced Polyarthritis in Rats—Changes in the Adjuvant-induced Paw [Test 1]

Drugs	Dose a) (mg/kg)	Number of animals	Volume of the paw injected with adjuvant (inhibition %)						
			15th day b)	16th day	17th day	18th day	19th day	22nd day	25th day
Solvent control group	0	6	3.36	3.51	3.49	3.29	3.46	3.72	3.33
Micro-emulsion containing methyl 4-biphenylyl-acetate	2.5	6	3.33	3.20(8.8)	2.91(16.6)	2.87 (12.8)	2.79* c) (19.4)	3.00* (19.4)	3.02(9.3)
	5.0	6	3.32	3.02(14.0)	2.93(16.0)	2.69* (18.2)	2.56** (26.0)	3.04* (18.3)	3.08(7.5)
	10.0	6	3.35	3.26 (7.1)	3.02(13.5)	2.76 (16.1)	2.58** (25.4)	3.05(18.0)	3.31(0.6)
Micro-emulsion containing ethyl 4-biphenylyl-acetate	2.5	6	3.36	3.23 (8.0)	3.17 (9.2)	3.05 (7.3)	2.97(14.2)	3.45(7.3)	3.36(−0.9)
	5.0	6	3.35	3.23 (8.0)	2.98(14.6)	2.81 (14.6)	2.70* (23.0)	3.16(15.1)	3.28(1.5)
	10.0	6	3.27	2.87(18.2)	2.89(17.2)	2.83 (14.0)	2.64* (23.7)	2.98* (19.9)	3.34(−0.3)

a): Calculated as 4-biphenylylacetic acid.

b): Days after injection of the injection (each test drug was intravenously administered for 5 days from the 15th to the 19th day.

c): Statistically analyzed figures against the solvent control group.
* $P<0.05$, ** $p<0.01$

[Test 2]

(a) Experimental animals:

SD-strain famale rats 140-180g)

(b) Test drugs:

The same as in A-1 [Test 2]

(c) Experimental procedure:

Rats were anesthetized with ether inhalation, and 0.6 mg of Mycobacterium butyricum suspended in 0.1 ml of white paraffin oil was immediately injected intradermally into the planter surface of the left hind paw of each rat via a 27 gauge needle fitted to a 0.5 ml syringe (day 0). On the 14th day after the adjuvant injection, arthritis-established rats selected by development of secondary lesions were used. Volumes of both hind paws in arthritis-established rats were measured by a plethysmometer (UGO BASILE), and the rats were randomized on the basis of the edema volume in the adjuvant-injected paw. Once daily, beginning on the 14th day up to the 18th day, drugs and vehicles were administered intravenously. Measurements of both paw volumes were performed every day from the 15th day up to the 18th day, and on the 21st day and the 24th day. The inhibition percent were calculated in accordance with the calculation formula as in [Test 1] of A-1.

(d) Experimental results

The micro-emulsions of this invention showed an excellent anti-inflammatory action on adjuvant-induced arthritis in rats in intravenous administration in doses of 1.25 to 10.0 mg/kg calculated as 4-biphenylylacetic acid.

The ED_{20} was 5.8 mg/kg calculated as 4-biphenylylacetic acid.

In contrast, when ethyl or sodium 4-biphenylylacetate was administered as an ordinary solution, its ED_{20} was 14.5 and 14.3 mg/kg, respectively, calculated as 4-biphenylylacetic acid.

Accordingly, the micro-emulsion of this invention has about 3 times as great an effect as an ordinary solution form of the active compound.

Table 4. Effects on Adjuvant-induced Polyarthritis in Rats-Changes in the Adjuvant-injected Paw [Test 2]

Test drugs	Dose (mg/kg) a)	No. of Animals	Inhibition (%) Days after Adjuvant Injection 15	16	17	18	21	24	ED_{20} (mg/kg) b)
Micro-emulsion of ethyl 4-biphenylyl-acetate	1.25 2.5 5.0 10.0	8 8 8 8	9.1 11.6 14.9** 21.3	10.3 14.6* 16.0* 23.4	16.4** c) 17.6** 25.8** 30.9	13.2* 16.1** 23.5** 27.9	6.2 5.7* 18.7 16.4	9.3 9.9 18.1 19.0	5.8
Ethyl 4-biphenylyl-acetate	1.25 2.5 5.0 10.0	8 8 8 8	-8.0 -3.7 5.6 2.8	-5.1 0 7.2 10.7	-3.3 6.7 15.5 15.2	-4.6 5.8 15.0 15.6	-19.2 -6.5 8.9 3.0	-23.2 -17.5 1.3 -9.8	14.5
Sodium 4-biphenylyl-acetate	1.25 2.5 5.0 10.0	7 8 8 8	-1.8 -4.2 7.3 8.2	1.2 1.5 12.9 9.5	7.9 1.9 17.9 17.9	3.9 -3.9 15.6 16.6	-7.5 -15.7 9.2 1.3	-14.1*** -29.7 2.8 -0.7	14.3
Aspirin® DL·Lysine	50 100 200	8 8 7	-2.7 4.0 6.7	-5.2 9.2 11.3	-3.8 13.2 16.4	-2.6 6.8 15.3	-12.4 4.2 7.2	-20.0** -2.8 6.2	320.5

a) Doses of the micro-emulsion of ethyl 4-biphenylylacetate, ethyl 4-biphenylylacetate and sodium 4-biphenylylacetate are expressed as those of 4-biphenylylacetic acid, and doses of aspirin® DL-lysine as those of aspirin®.

b) Dose required to cause a 20% inhibition of adjuvant-injected paw volume against the vehicle control. This value was calculated from the mean % inhibition of swelling combined for measurement intervals indicated.

c) * $P<0.05$, ** $p<0.01$

A-3: Analgesic Activity (acetic acid stretching method)

(a) Experimental animals:

dd-strain male mice (body weight about 18 g), 10 per group

(b) Test drugs:

Micro-emulsions containing 2 % (w/v), calculated as 4-biphenylylacetic acid, of methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate respectively (prepared as in Examples 1 and 2) were each diluted with Venolipid® to 4, 8, and 16 times, and administered at a dose of 0.1 ml/10g of body weight. Venolipid® was used as a vehicle control.

(c) Experimental procedure

Each of the test drugs was administered into the tail vein of each mouse, and 5 minutes later, 0.6% acetic acid (0.1 ml/10 g) was intraperitoneally administered. The number of stretchings for 10 minutes was measured, and the inhibition rate against the vehicle control group was calculated.

(d) Experimental Results

The results are shown in Figure 1. When acetic acid was intraperitoneally administered after the administration of each test drug into the tail vein of the rat, the 50% inhibition rates (ED_{50}) of the micro-emulsion containing methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate were 18 mg/kg and 23 mg/kg respectively. In either case, the analgesic effect was noted.

A-4: Effects on reversal of abnormal 3-legged gait in rats (analgesic activity)

(a) Experimental animals:

SD-strain male rats (120-165g)

(b) Test drugs:

The same as in A-1 [Test 2]

(c) Experimental procedure:

A 40% suspension of dried brewers yeast in physiological saline was injected (0.25 ml/rat) subcutaneously into the planter surface of the left hind paw of each rat. Three hours later, the walking gait on a wire mesh platform was assessed for each rat.

Gist scoring system:
Score of:

- 0 = Normal gait in the presence of a severely inflamed paw. There is continuous use of the inflamed foot pad.
- 0.5 = As above, with intermittent mild limping.
- 1.0 = Constant limping, but continuous use of the inflamed foot pad.
- 1.5 = Limping with occasional 3-legged gait (paw kept off walking surface) or intermittent use of digits in combination with the inflamed foot pad.
- 2.0 = Continous 3-Legged gait and/or only the tips of the digits touching the waling surface without using the inflamed foot pad.

Any rat not showing a gait score of 2 was eliminated from this test. Drugs and vehicles were then administered intravenously. Measurements of the gait were made at 1, 2, 3 and 4 hours after drug administration.

(d) Experimental results:

The micro-emulsions of this invention showed an effect on an abnormal gait of the inflamed paw in

dos s of 2.5 to 10.0 mg/kg calculated as 4-biphenylylacetic acid, and its ED_{50} value was 4.8 mg kg calculated as 4-biph nylylacetic acid.

In contrast, sodium 4-biphenylacetate in an ordinary solution form showed an ED_{50} of 7.1 mg kg. Accordingly, the effect of the micro-emulsion of this invention was especially superior.

Table 5. Effects on reversal of abnormal 3-legged gait in rats

Test drugs	No. of animals	ED_{50} a) (mg/kg)
Sodium 4-biphenylylacetate	7	7.1
Micro-emulsion of ethyl 4-biphenylylacetate (Example 2)	7	4.8
Aspirin®-DL•Lysine	7	93.2

a) Dose required to cause at least a 50% reversal of gait score of 2(<=1 score) in 50% of the rats. This value was calculated from the highest effective rate for measurement intervals.

A-5: Effects on body temperature in pyretic rats antipyretic activity

(a) Experimental animals:

SD-strain male rats (120-185g)

(b) Test drugs:

The same as in A-1 [Test 2]

(c) Experimental procedure:

Male Sprague-Dawley rats, weighing 120-185g, were injected subcutaneously in the nape of the neck with 2 ml of a 20% suspension of dried brewers yeast in physiological saline. Drugs and vehicles were administered intravenously 17 hours after treatment of yeast suspension. Rectal temperature was recorded with a thermistor type thermometer (Natsume Seisakusho, BMA-77 Type) just prior to drug administration and at 1/2, 1, 2 and 4 hours after drug administration.

(d) Experimental resutls:

The micro-emulsions of this invention showed a significant antipyretic activity on brewer's yeast-induced fever in doses of 1.25 to 10.0 mg/kg calculated as 4-biphenylylacetic acid. Ethyl or sodium 4-biphenylylacetate in an ordinary solution form also showed a significant antipyretic activity in doses of 2.5 to 10.0 mg/kg, calculated as 4-biphenylylacetic acid.

The ED_{50} of the micro-emulsion of this invention was 1.5 mg kg, whereas ethyl 4-biphenylylacetate and

sodium 4-biphenylylacetate showed an ED_{50} of 3.1 and 2.6 mg/kg.

It is found therefore that the micro-emulsion of this invention has 2 times as good an effect as the ordinary solution forms of ethyl and sodium 4-biphenylylacetates.

Table 6. Antipyretic effects

Test drugs	No. of animals	ED_{50} a) (mg/kg)
Micro-emulsion of ethyl 4-biphenylylacetate	9	1.5
Ethyl 4-biphenylylacetate	10	3.1
Sodium 4-biphenylylacetate	9	2.6
Aspirin®-DL•Lysine	9	76.3

a) Dose required to reduce a body temperature by at least 1.5°C against the vehicle control in 50% of the rats. This value was calculated from the highest effective rate for measurement intervals.

[B] Distribution to the tissue

(a) Experimental animals:

Wistar-strain male rats (body weight 60-220 g), 6 per group.

(b) Test drugs:

A micro-emulsion containing 2 % (w/v), calculated as 4-biphenylylacetic acid, of methyl 4-biphenylylacetate (prepared as in Example 1) and an aqueous solution of sodium 4-biphenylylacetate as a control drug.

(c) Experimental procedure:

The test drug was administered into the tail vein in a dose of 10 mg/kg, calculated as 4-biphenylylacetic acid. The rats were periodically killed, and the major organs were extracted. The concentration of the drug in the tissues were measured by the conventional HPLC method. The concentration within the tissue was calculated as 4-biphenylylacetic acid.

(d) Experimental results:

The results are shown in Figure 2. The distribution of the methyl 4-biphenylylacetate and sodium 4-biphenylylacetate to the tissue were examined. At 30 seconds after the administration of the test drugs, both

drugs reached concentrations above those at which they showed a pharmacological activity (the action of 4-biphenylylacetic acid to inhibit synthesis of prostaglandin: IC_{50} 0.68 ng.ml (see E. L. Tolman. American Cyanamid Company's Reprot). It is seen that in the spleen and in the muscles, the micro-emulsion containing methyl 4-biphenylylaceate was distributed in a concentration 2.5 to 3 times as high as sodium 4-biphenylylacetate. This suggests that micro-emulsion containing methyl 4-biphenylylacetate transfers to the tissues within a shorter period of time, and its effect in a lesser dose is expected. Ethyl 4-biphenylylacetate showed the same transferability to the tissues, and exhibits an excellent effect.

[C] Toxicity test

Charles River-strain SD male rats (6 weeks old; body weight 160-170 g), 5 per group, were used. A micro-emulsion containing 2 % (w/v), calculated as 4-biphenylylacetic acid, of methyl 4-biphenylylacetate or ethyl 4-biphenylylacetate (prepared in accordance with Example 1 or 2 given hereinbelow) was administered once into the tail vein. The animals were observed for 3 days to perform a toxicity test. The doses were 50, 100, 200 and 400 mg/kg. As a control, an aqueous solution of sodium 4-biphenylylacetate was administered. The results are shown in Table 7.

From the results no change ascribable to the drugs was noted in the observations of general symptoms and the results of autopsy when the micro-emulsions containing methyl 4-biphenylylacetate and ethyl 4-biphenylylacetate were administered in doses of up to 100 mg.kg. On the other hand, with the control sodium 4-biphenylylacetate, no change in general symptoms and the results of autopsy was noted in administration in doses of up to 50 mg.kg. But changes occurred in administration of more than 100 mg.kg. Accordingly, it is evident that the micro-emulsion of this ivnention is safer than sodium 4-biphenylylacetate in the ordinary solution form.

Table 7 Toxicity Test

Drug	Dose (a) (mg/kg)	Amount admini-stered (ml/kg)	Number of the dead	Findings: General symptoms	Findings: Autopsy
Solvent control group	0	20	0/5	No change	No change
Micro-emulsion containing methyl 4-biphenylyl-acetate (2%, w/v) (Example 1)	50 100	2.5 5	0/5 0/5	No change	No change
	200 400	10 20	0/5 1/5	Body weight increase inhibition (at least 200 mg/kg), spontaneous motion decreased (400 mg/kg), pallor (400 mg/kg)	Peptic ulcer occurred (at least 200 mg/kg), fibrinogenous intestinal adherence (at least 200 mg/kg)
Micro-emulsion containing ethyl 4-biphenylyl-acetate (2%, w/v) (Example 2)	50 100	2.5 5	0/5 0/5	No change	No change
	200 400	10 20	0/5 1/5	Body weight increase inhibition (at least 200 mg/kg), spontaneous motion decreased (400 mg/kg), pallor (400 mg/kg)	Peptic ulcer occurred (at least 200 mg/kg), fibrinogenous intestinal adherence (at least 200 mg/kg)
Sodium 4-biphenylyl-acetate	50	2.5	0/5	No change	No change
	100 200 400	5 10 20	0/5 3/5 3/5	Body weight increase inhibition (at least 100 mg/kg), spontaneous motion decreased (100 mg/kg), tarry feces (200 mg/kg), pallor (at least 200 mg/kg)	Peptic ulcer occurred (at least 100 mg/kg), fibrinogenous intestinal adherence (at least 100 mg/kg)

(a) Calculated as 4-biphenylylacetic acid.

[D] Stability test:

The micro-emulsions containing the 4-biphenylacetic acid ester provided by this invention were tested for 6 months for stability. The content was measured by high-performance liquid chromatography (device: 655-15 made by Hitachi Limited), and the particle size was measured by a light-transmission type particle

size distribution analyzer (CAPA-500, made by Horiba Limited). The results are shown in Table 8. In a stability test at room temperature (25 °C) for 6 months, no change was observed in content, appearance, pH and particle diameter. Accordingly, the micro-emulsion of this invention is very stable pharmaceutically.

Table 8

Stability Test

Micro-emulsion	Test items	Period of observation: Immediately after preparation	1 month	3 months	6 months
Example 1	Content (mg/ml) (residual rate, %)	22.08 (100.0)	22.30 (101.0)	21.86 (99.0)	21.86 (99.0)
	Appearance	White non-transparent emulsion	-	-	-
	pH	6.80	6.62	6.73	6.63
	Mean particle diameter (μm)	0.15	0.16	0.17	0.16
Example 2	Content (mg/ml) (residual rate, %)	23.12 (100.0)	23.06 (99.7)	23.20 (100.3)	23.00 (99.5)
	Appearance	White non-transparent emulsion	-	-	-
	pH	6.65	6.58	6.54	6.50
	Mean particle diameter (μm)	0.15	0.16	0.17	0.15
Example 3	Content (mg/ml) (residual rate, %)	21.20 (100.0)	21.14 (99.7)	21.24 (100.2)	21.14 (99.7)
	Appearance	White non-transparent emulsion	-	-	-
	pH	6.42	6.38	6.36	6.34
	Mean particle diameter (μm)	0.16	0.17	0.16	0.14
Example 15	Content (mg/ml) (residual rate, %)	32.15 (100.0)	31.61 (98.3)	31.53 (98.1)	31.84 (99.0)
	Appearance	White non-transparent emulsion	-	-	-
	pH	6.81	6.69	6.89	6.78
	Mean particle diameter (μm)	0.12	0.12	0.12	0.12
Example 16	Content (mg/ml) (residual rate, %)	46.80 (100.0)	47.03 (100.5)	46.41 (99.2)	46.40 (99.1)
	Appearance	White non-transparent emulsion	-	-	-
	pH	6.81	6.91	6.80	6.93
	Mean particle diameter (μm)	0.13	0.13	0.13	0.13

[E] Clinical tests

(a) Test drugs:

Lipo BPAA: Micro-emulsion containing ethyl 4-biphenylylacetate prepared in Example 2: administered

once in a dose of 40 mg/2 ml, calculated as 4-biphenylylacetic acid, by intravenous injection.

Decadron®: sodium dexamethasone phosphate, a steroidal anti-inflammatory drug produced by Merck-Banyu Company, administered once in a dose of 4 mg/1 ml

Venopyrin®: aspirin®DL-lysine, a salicylate-type preparation made by Green Cross Company; 900 mg

(b) Method of Evaluation:

Self-evaluation by patients in accordance with 10-step numerical scale for pain degree

10: severest pain
5: moderate pain
0: no pain

(c) Experimental results

Figure 3 shows the 10 step scales of the analgesic effects of the micro-emulsion of this invention (Lipo BPAA) and Decadron® in patients with neuralgia. Figure 4 shows the 10 step scales of the analgesic effects of Lipo BPAA and Decadron® on patients with calculosis. Figure 5 shows the 10-step scales of lipo BPAA and Venopyrine® as the degree of alleviation of pain in patients with chronic rheumatoid arthritis.

Figures 6 and 7 showed the 10-step scales of Lipo BPAA as the degree of allevation of pain in patiens with acute upper respiratory tract inflammation and patients with cancerous pain.

As is clear from the results shown in Figures 3 to 7, the micro-emulsion of this invention had a marked effect on urolithic pain and neuralgia on which steroidal agents show little or no effect, and showed higher effectiveness on chronic rheumatoid arthritis than Venopyrin®. It also showed a marked effect on pharyngalgia of a patient with acute upper respiratory tract inflammation. The analgesic effect of the micro-emulsion of this invention is characterized by its rapid manifestation and durability. Particularly, sialolithiasis pain was completely removed in 10 to 20 minutes after injection.

The micro-emulsion of this invention showed no side effects.

As stated above, the micro-emulsion of this invention containing the 4-biphenylylacetic acid ester has excellent distribution (incorporation to a site of inflammation with reduced side effects and toxicity). The pharmacological effect of the active ingredient of the micro-emulsion is exhibited effectively and strongly over an extended period of time. Furthermore, since the micro-emulsion is stable, it is very useful clinically as a liquid injectable preparation for anti-inflammatory, analgesic and antipyretic purposes.

Injection (parenteral, e.g., intravenous, intra-articular, etc.), eye-dropping, etc. can be cited as routes of administration of this micro-emulsion. The dosage varies according to the administration route, prescription, patient's symptoms, etc., but a usual dose for adults is 5 mg to 50 mg (as 4-biphenylylacetic acid) each time, once to three times a day. This dosage, of course, can be exceeded according to the severity of the condition, body weight, sex and type of disease of a patient, the physician's judgement, etc. The administration of this emulsion brings about a marked improvement in rheumatoid arthritis, osteoarthritis, lumbago, frozen shoulder, neck/shoulder/arm syndrome, post-operative and traumatic inflammation and pain, cancerous pain, herpes zoster, gout attack, tendinitis/ tenosynovitis, neuralgia, myalgia, pain after tooth extraction, conjunctivitis, uveitis, etc.

The following Examples illustrate the present invention more specifically.

EXAMPLE 1

4.28 g (corresponding to 4.0 g of 4-biphenylylacetic acid) of methyl 4-biphenylylacetate was added to 20 g of soybean oil described in Japanese Pharmacopoeia, and the mixture was dissolved under heat. Then, 2.4 g of purified soybean phospholipid and 5 g of glycerol were added to the solution, and the mixture was vigorously stirred under heat. A suitable amount of distilled water was added, and the mixture was stirred by a polytron® homogenizer to prepare a crude emulsion. The crude emulsion was emulsified under high pressure by a Gaulin high-energy type homogenizer, and distilled water was added to adjust the amount of the emulsion to 200 ml. There was obtained a micro-emulsion containing methyl 4-biphenylylacetate. The dispersed lipid particles had a mean particle diameter of 0.15 μm, and it did not contain particles having a size of at least 1 μm.

EXAMPLE 2

Example 1 was repeated except that 4.52 g of ethyl 4-biphenylylacetate was used instead of 4.28 g of

methyl 4-biphenylylacetate. Thus, a micro-emulsion containing ethyl 4-biphenylylacetate was obtained.

The dispersed lipid particles in the micro-emulsion had a mean particle diameter of 0.15 μm. and it did not contain particles having a size of at least 1 μm.

EXAMPLE 3

Example 1 was repeated except that purified yolk phospholipid was used instead of the purified soybean phospholipid. A micro-emulsion containing methyl 4-biphenylylacetate was obtained.

EXAMPLE 4

Ethyl 4-biphenylylacetate (0.43 g corresponding to 0.4 g of 4-biphenylylacetic acid) was added to 20 g of soybean oil described in the Japanese Pharmacopoeia. and the mixture was heated to form a solution. To the solution were added 2.0 g of Pluronics F-68® (a polyoxyethylene polyoxypropylene ether-type nonionic surface-active agent made by Asahi-Denka Kogyo K. K.) and a suitable amount of distilled water. The mixture was stirred by a polytron® homogenizer to preapre a crude emulsion. The crude emulsion was emulsified under high pressure by a Gaulin high-energy homogenizer. and distilled water was added to make 200 ml. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed lipid particles in the micro-emulsion had a mean diameter of 0.15 μm. and it did not contain particles having a size of at least 1 μm.

EXAMPLE 5

Ethyl 4-biphenylylacetate (0.43 g) was added to 10 g of soybean oil described in the Japanese Pharmacopoeia and 10 g of MCT, and dissolved under heat. Purified soybean phospholipid (1.2 g), 1.2 g of purified yolk phospholipid and 5 g of glycerol were added, and the mixture was vigorously stirred under heat. After dissolving, a suitable amount of water was added, and the mixture was stirred by a polytron®homogenizer to form a crude emulsion. The crude emulsion was emulsified under high pressure by a Gaulin high-energy homogenizer. Distilled water was added to make 200 ml. A micro-emulsion containing ethyl 4-biphenylylacetate as obtained. The dispersed lipid particles in the micro-emulsion had a mean particle diameter of 0.15 μm, and it did not contain particles having a size of at least 1 μm.

EXAMPLE 6

Example 1 was repeated except that the amounts of soybean oil. methyl 4-biphenylylacetate. purified soybean phospholipid, and glycerin were changed to 100 g. 53.5 g. 7.5 g and 12.5 g, respectively. There was obtained 250 ml of a micro-emulsion containing methyl 4-biphenylylacetate in a high concentration. The dispersed lipid particles in the micro-emulsion had a mean particle diameter of 0.15 μm. and it did not contain particles having a size of at least 1 μm.

EXAMPLE 7

Ethyl 4-biphenylylacetate (0.43 g) was dissolved in 20 g of soybean oil described in the Japanese Pharmacopoeia under heat. To the solution were added 2.4 g of purified soybean phospholipid. 0.6 g of cholesterol and 5 g of glycerol, and the mixture was vigorously stirred under heat. A suitable amount of distilled water was added, and the mixture was stirred by a Polytoron® homogenizer to prepare a crude emulsion and then emulsified under high pressure by a Gaulin high-energy homogenizer. Distilled water was added to make 200 ml. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed lipid particles in the micro-emulsion had a mean particle size of 0.14 μm. and it did not contain particles having a size of at least 1 μm.

EXAMPLE 8

Example 7 was repeated except that 0.04 g of sodium palmitate was added instead of 0.6 g of cholesterol. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained.

EXAMPLE 9

Example 7 was repeated except that 0.4 g of O-palmitoyl dextran (molecular weight 40000) was added instead of 0.6 g of cholesterol. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained.

EXAMPLE 10

Albumin (5 g) was added to the micro-emulsion obtained in Example 1, and the mixture was lyophilized to obtain a powder of a micro-emulsion of containing ethyl 4-biphenylylacetate.

EXAMPLE 11

Ethyl 4-biphenylylacetate (0.2825 g corresponding to 0.25 g of 4-biphenylylacetic acid) was added to 100 g of soybean oil described in the Japanese Pharmacopoeia, and dissolved under heat. To the solution were added 24 g of purified soybean phospholipid and 50 g of glycerol, and the mixture was vigorously stirred under heat. A suitable amount of distilled water was added to the solution, and the mixture was stirred by a polytron® homogenizer to prepare a crude emulsion.

The crude emulsion was then emulsified under high pressure by a Gaulin®high-energy homogenizer, and distilled water was added to make 1000 ml. A micro-emulsion containing ethyl 4-biphynylylacetate was obtained. The dispersed particles in the micro-emulsion had a mean particle diameter of 0.16 μm, and it did not contain particles with a particle diameter of at least 1 μm.

EXAMPLE 12

Example 11 was repeated except that the amount of ethyl 4-biphenylylacetate was changed to 0.565 g. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed lipid particles in the micro-emulsion had a mean particle diameter of 0.18 μm, and it did not contain particles having a size of at least 1 μm.

EXAMPLE 13

Example 11 was repeated except that the amount of ethyl 4-biphenylylacetate was changed to 1.13 g. A micro-emulsion containing ethyl 4-biphenylylacetate was prepared. The dispersed lipid particles in the micro-emulsion had a mean particle diameter of 0.14 μm, and it did not contain particles having a size of at least 1 μm.

EXAMPLE 14

Example 11 was repeated except that the amount of ethyl 4-biphenylylacetate was changed to 2.26 g. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed particles in the micro-emulsion had a mean particle diameter of 0.13 μm, and it did not contain particles having a size of at least 1 μm.

EXAMPLE 15

Example 11 was repeated except that the amount of ethyl 4-biphenylylacetate was changed to 33.9 g. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed particles in the micro-emulsion had an average particle diameter of 0.13 μm, and it did not contain particles having a size of at least 1 μm.

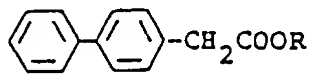
EXAMPLE 16

Example 11 was repeated except that the amount of ethyl 4-biphenylylacetate was changed to 45.2 g. A micro-emulsion containing ethyl 4-biphenylylacetate was obtained. The dispersed particles in the micro-emulsion had a mean particle diameter of 0.13 μm, and it did not contain particles having a size of at least 1 μm.

Claims

1. A pharmaceutical oil-in-water type micro-emulsion comprising fine particles having a mean particle

diameter of from 0.1 to 1.0 μm of a vegetable oil or a triglyceride of a medium-chain fatty acid having 8 to 12 carbon atoms containing 0.01 to 10% (w/v) of a 4-biphenylylacetic acid ester of the formula



(I)

wherein R represents an alkyl group having 1 to 18 carbon atoms, an aqueous medium, and 0.05 to 25% (w/v) of a physiologically acceptable phospholipid for dispersing said fine particles in said aqueous medium.

2. The micro-emulsion of claim 1 wherein the 4-biphenylylacetic acid ester is ethyl 4-biphenylylacetate.

3. The micro-emulsion of claim 1 wherein the vegetable oil is pharmaceutically acceptable soybean oil

4. The micro-emulsion of claim 1 wherein the physiologically acceptable phospholipid is a purified vegetable oil phospholipid.

5. The micro-emulsion of claim 4 wherein the purified vegetable oil phospholipid is purified soybean oil phospholipid.

6. The micro-emulsion of claim 1 which further contains an isotonizing agent selected from the group consisting of glycerol, sugar alcohols, monosaccharides, disaccharides and amino acids.

7. The micro-emulsion of claim 1 which consists essentially of 5 to 50% (w v) of fine particles of a vegetable oil or a triglyceride of a medium-chain fatty acid having 8 to 12 carbon atoms containing 0 01 to 10% (w/v) of the 4-biphenylylacetic acid ester, 0.05 to 25% (w v) of the physiologically acceptable phospholipid, an isotonizing agent selected from the group consisting of glycerol. sugar alcohols. monosaccharides, disaccharides and amino acids in an amount sufficient to isotonize the emulsion, and water.

8. The micro-emulsion of claim 7 wherein the 4-biphenylylacetic acid ester is ethyl 4-biphenylylacetate.

9. The micro-emulsion of claim 1 which consists essentially of 5 to 30% (w v) of fine particles of soybean oil having dissolved therein 1 to 5% (w/v) of ethyl 4-biphenylylacetate, 0.5 to 25% (w v) of a purified soybean oil phospholipid, 1 to 5% (w/v) of glycerol, and the remainder being water.

10. The micro-emulsion of claim 1 wherein the fine particles of the oil or fat have a mean particle diameter of not more than 0.3 μm.

11. The micro-emulsion of claim 5 wherein the purified soybean oil phospholipid has a phosphatidyl choline content of at least 80% and an iodine value of 35 to 45.

12. The pharmaceutical oil-in-water type micro-emulsion of any one of claims 1 to 11 for use in the treatment of inflammation, pain and/or fever in a mammal.

13. A process for producing a pharmaceutical oil-in-water type micro-emulsion of any one of claims 1 to 11, which comprises dissolving a predetermined amount of said 4-biphenylylacetic acid ester in said vegetable oil or triglyceride under heat, adding a predetermined amount of the phospholipid and as required other additives, stirring the mixture to form a uniform mixture. then adding water. treating the mixture with a dispersion homogenizer to prepare an oil-in-water type crude emulsion. and thereafter homogenizing the crude emulsion by a high-energy homogenizer.

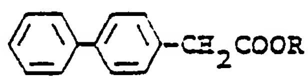
14. The method of claim 13 wherein the 4-biphenylylacetic acid ester is ethyl 4-biphenylylacetate.

15. The method of claim 13 wherein the crude emulsion is homogenized until the particles of the vegetable

oil or triglyceride attain a mean particle diameter of not more than 1 μm, preferably not more than 0.3 μm.

Revendications

1. Micro-émulsion pharmaceutique du type LH (huile-dans-l'eau) renfermant :
   - de fines particules, d'un diamètre moyen de 0,1 à 1,0 μm, d'une huile végétale ou d'un triglycéride dérivant d'un acide gras de longueur de chaîne moyenne, à 8-12 atomes de carbone, contenant de 0,01 à 10% (poids/volume) d'un ester de l'acide (biphénylyl-4)-acétique répondant à la formule :



(I)

   dans laquelle R représente un radical alkyle contenant de 1 à 18 atomes de carbone,
   - un milieu aqueux et
   - de 0,05 à 25% (poids/volume) d'un phospholipide physiologiquement acceptable dont la fonction est de disperser lesdites fines particules dans ledit milieu aqueux.

2. Micro-émulsion selon la revendication 1 dans laquelle l'ester de l'acide (biphénylyl-4)-acétique est le (biphénylyl-4)-acétate d'éthyle.

3. Micro-émulsion selon la revendication 1 dans laquelle l'huile végétale est une huile de soja acceptable du point de vue pharmaceutique.

4. Micro-émulsion selon la revendication 1 dans laquelle le phospholipide physiologiquement acceptable est un phospholipide d'huile végétale purifié.

5. Micro-émulsion selon la revendication 4 dans laquelle le phospholipide d'huile végétale purifié est le phospholipide d'huile de soja purifié.

6. Micro-émulsion selon la revendication 1 qui contient en outre un agent isotonisant pris dans l'ensemble constitué par le glycérol, les alcools de sucres, les monosaccharides, les disaccharides et les amino-acides.

7. Micro-émulsion selon la revendication 1 qui est essentiellement constituée :
   - de 5 à 50% (poids/volume) de fines particules d'une huile végétale ou d'un triglycéride dérivant d'un acide gras de longueur de chaîne moyenne, ayant de 8 à 12 atomes de carbone, particules qui contiennent de 0,01 à 10% (p/v) d'un ester de l'acide (biphénylyl-4)-acétique,
   - de 0,05 à 25% (p/v) du phospholipide physiologiquement acceptable,
   - d'un agent isotonisant choisi dans l'ensemble constitué par le glycérol, les alcools de sucres, les monosaccharides, les disaccharides et les aminoacides, en une quantité suffisante pour rendre l'émulsion isotonique, et
   - d'eau.

8. Micro-émulsion selon la revendication 7 dans laquelle l'ester de l'acide (biphénylyl-4)-acétique est le (biphénylyl-4)-acétate d'éthyle.

9. Micro-émulsion selon la revendication 1 qui est essentiellement constituée :
   - de 5 à 30% (p/v) de fines particules d'huile de soja contenant, en solution, de 1 à 5% (p/v) de (biphénylyl-4)-acétate d'éthyle,
   - de 0,5 à 25% (p/v) d'un phospholipide d'huile de soja purifié,
   - de 1 à 5% (p/v) de glycérol et

- d'eau pour le reste.

10. Micro-émulsion selon la revendication 1 dans laquelle les fines particules de l'huile ou de la graisse ont un diamètre particulaire moyen d'au plus 0,3 μm

11. Micro-émulsion selon la revendication 5 dans laquelle le phospholipide d'huile de soja purifié a une teneur en phosphatidyl-choline d'au moins 80% et un indice d'iode de 35 à 45.

12. Micro-émulsion pharmaceutique du type LH selon l'une quelconque des revendications 1 à 11 pour ses emplois dans le traitement de l'inflammation, de la douleur et ou de la fièvre chez un mammifère.

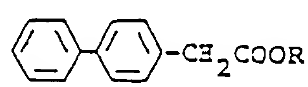
13. Procédé de préparation d'une micro-émulsion pharmaceutique du type LH selon l'une quelconque des revendications 1 à 11, procédé selon lequel on dissout, en chauffant, une quantité donnée de l'ester de l'acide (biphénylyl-4)-acétique dans l'huile végétale ou le triglycéride, on ajoute une quantité donnée du phospholipide et, si cela est nécessaire, d'autres additifs, on agite le mélange de manière à obtenir un mélange uniforme, puis on ajoute de l'eau, on traite le mélange à l'aide d'un homogénéiseur de dispersion pour préparer une émulsion brute du type LH (huile-dans-l'eau), et ensuite on homogénéise l'émulsion brute au moyen d'un homogénéiseur de grande énergie.

14. Procédé selon la revendication 13 dans lequel l'ester de l'acide (biphénylyl-4)-acétique est le (biphénylyl-4)-acétate d'éthyle.

15. Procédé selon la revendication 13 dans lequel l'émulsion brute est homogénéisée jusqu'à ce que les particules de l'huile végétale ou du triglycéride aient atteint un diamètre particulaire moyen d'au plus 1 μm, de préférence d'au plus 0.3 μm.

Ansprüche

1. Pharmazeutische Mikroemulsion des Öl-in-Wasser-Typs, dadurch **gekennzeichnet**, daß sie feine Teilchen mit einem mittleren Teilchendurchmesser von 0,1 bis 1.0 μm eines Pflanzenöls oder eines Triglycerids einer Fettsäure mit mittlerer Kette und 8 bis 12 Kohlenstoffatomen, die 0.01 bis 10% (Gew./Vol.) eines 4-Biphenylyl-essigsäureesters der Formel



(I)

worin R eine Alkylgruppe mit 1 bis 18 Kohlenstoffatomen bedeutet, enthalten, ein wäßriges Medium und 0,05 bis 25% (Gew./Vol.) eines physiologisch annehmbaren Phospholipids für die Dispersion der feinen Teilchen in dem wäßrigen Medium enthält.

2. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß der 4-Biphenylyl-essigsäureester Ethyl-4-biphenylylacetat ist.

3. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß das Pflanzenöl ein pharmazeutisch annehmbares Sojabohnenöl ist.

4. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß das physiologisch annehmbare Phospholipid ein gereinigtes Pflanzenöl-Phospholipid ist.

5. Mikroemulsion nach Anspruch 4, dadurch **gekennzeichnet**, daß das gereinigte Pflanzenöl-Phospholipid gereinigtes Sojabohnenöl-Phospholipid ist.

6. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß sie zusätzlich ein Isotonisierungsmittel, ausgewählt aus der Gruppe Glycerin, Zuckeralkohol, Monosaccharide, Disaccharide und Aminosäu-

ren, enthält.

7. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß sie im wesentlichen aus 5 bis 50% (Gew./Vol.) feiner Teilchen aus einem Pflanzenöl oder eines Triglycerids einer Fettsäure mit mittlerer Kette mit 8 bis 12 Kohlenstoffatomen, die 0,01 bis 10% (Gew./Vol.) 4-Biphenylyl-essigsäureester enthalten, 0,05 bis 25% (Gew./Vol.) eines physiologisch annehmbaren Phospholipids, ein Isotonisierungsmittel, ausgewählt aus der Gruppe Glycerin, Zuckeralkohol, Monosaccharide, Disaccharide und Aminosäuren, in einer Menge, die ausreicht, die Emulsion zu isotonisieren, und Wasser besteht.

8. Mikroemulsion nach Anspruch 7, dadurch **gekennzeichnet**, daß der 4-Biphenylyl-essigsäureester Ethyl-4-biphenylylacetat ist.

9. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß sie im wesentlichen aus 5 bis 30% (Gew./Vol.) feiner Teilchen aus Sojabohnenöl, das darin gelöst 1 bis 5% (Gew./Vol.) Ethyl-4-biphenylylacetat enthält, 0,5 bis 25% (Gew./Vol.) gereinigten Sojabohnenölphospholipid, 1 bis 5% (Gew./Vol.) Glycerin und als Rest Wasser besteht.

10. Mikroemulsion nach Anspruch 1, dadurch **gekennzeichnet**, daß die feinen Teilchen aus Öl oder Fett einen mittleren Teilchendurchmesser von nicht über 0,3 μm aufweisen.

11. Mikroemulsion nach Anspruch 5, dadurch **gekennzeichnet**, daß das gereinigte Sojabohnenölphospholipid einen Phosphatidyl-cholin Gehalt von mindestens 80% und eine Jodzahl von 35 bis 45 besitzt.

12. Pharmazeutische Mikroemulsion des Öl-in-Wasser-Typs nach irgendeinem der Ansprüche 1 bis 11 für die Verwendung bei der Behandlung von Entzündungen, Schmerz und/oder Fieber in Säugetieren.

13. Verfahren zur Herstellung einer pharmazeutischen Mikroemulsion des Öl-in-Wasser-Typs nach irgendeinem der Ansprüche 1 bis 11, dadurch **gekennzeichnet**, daß eine vorbestimmte Menge des 4-Biphenylyl-essigsäureesters in dem Pflanzenöl oder Triglycerid unter Erwärmen gelöst wird, eine vorbestimmte Menge eines Phospholipids und gegebenenfalls andere Zusatzstoffe zugegeben werden, das Gemisch unter Bildung einer einheitlichen Mischung gerührt wird, dann Wasser zugegeben wird, das Gemisch mit einer Dispersions-Homigenisierungs-Vorrichtung behandelt wird, um eine rohe Emulsion des Öl-in-Wasser-Typs herzustellen, und anschließend die rohe Emulsion mittels eines Hoch-Energie-Homogenisierungs-Vorrichtung homogenisiert wird.

14. Verfahren nach Anspruch 13, dadurch **gekennzeichnet**, daß der 4-Biphenylyl-essigsäureester Ethyl-4-Biphenylylacetat ist.

15. Verfahren nach Anspruch 13, dadurch **gekennzeichnet**, daß die rohe Emulsion homogenisiert wird bis die Teilchen aus Pflanzenöl oder Triglycerid einen mittleren Teilchendurchmesser von nicht mehr als 1 μm, bevorzugt nicht mehr als 0,3 μm, annehmen.

FIG. 1

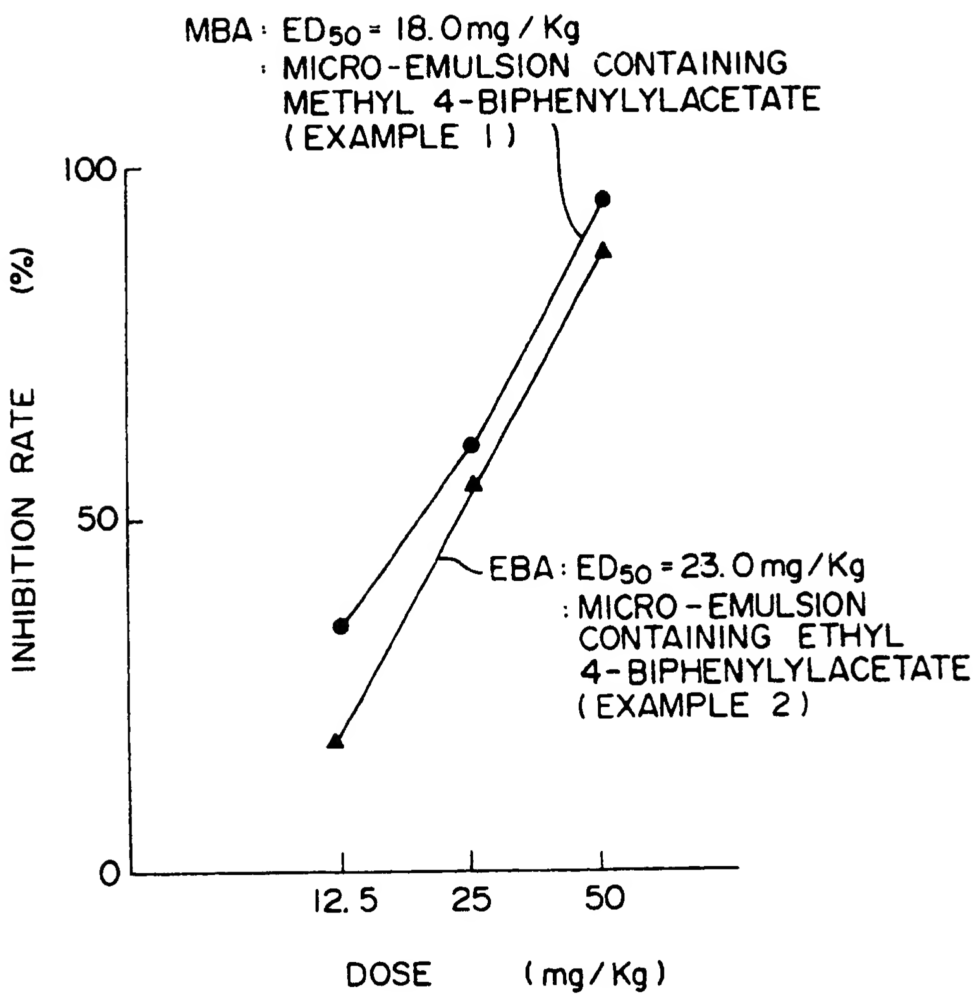

MBA : ED50 = 18.0 mg / Kg
: MICRO-EMULSION CONTAINING METHYL 4-BIPHENYLYLACETATE (EXAMPLE 1)
EBA : ED50 = 23.0 mg / Kg
: MICRO-EMULSION CONTAINING ETHYL 4-BIPHENYLYLACETATE (EXAMPLE 2)
INHIBITION RATE (%)
100
50
0
12.5
25
50
DOSE (mg / Kg)

FIG. 2

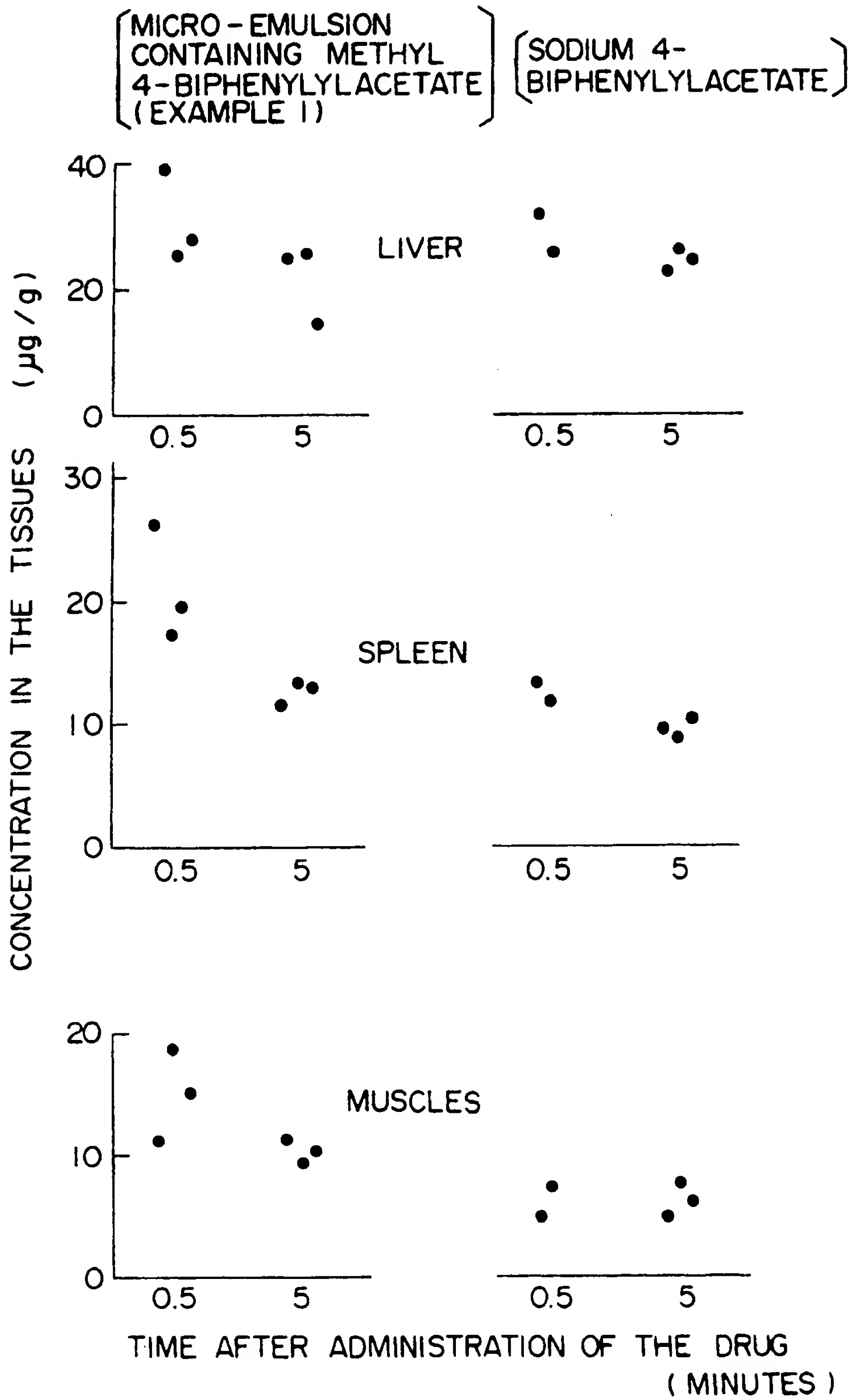

MICRO-EMULSION CONTAINING METHYL 4-BIPHENYLYLACETATE (EXAMPLE 1)
SODIUM 4-BIPHENYLYLACETATE
CONCENTRATION IN THE TISSUES (μg / g)
LIVER
SPLEEN
MUSCLES
40
20
0
30
20
10
0
20
10
0
0.5
5
0.5
5
TIME AFTER ADMINISTRATION OF THE DRUG (MINUTES)

FIG. 3 ANALGESIC EFFECTS OF LIPO BPAA AND DECADRON® ON VARIOUS KINDS OF NEURALGIA (CROSSOVER METHOD)

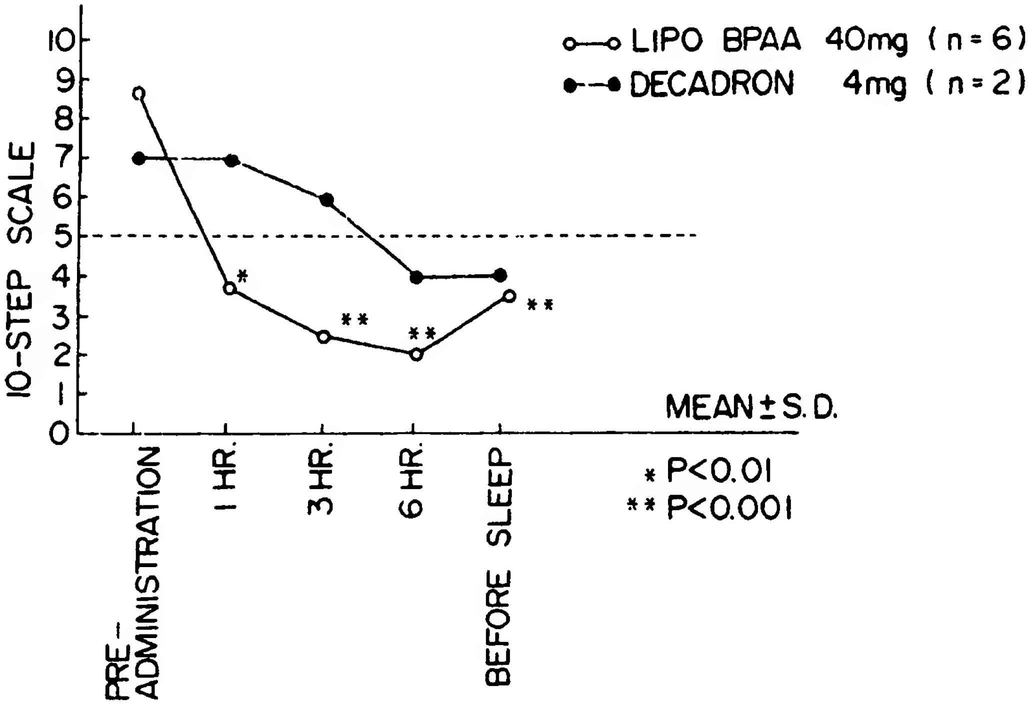


FIG. 4 ANALGESIC EFFCTS OF LIPO BPAA AND DECADRON® ON CALCULUS PAIN

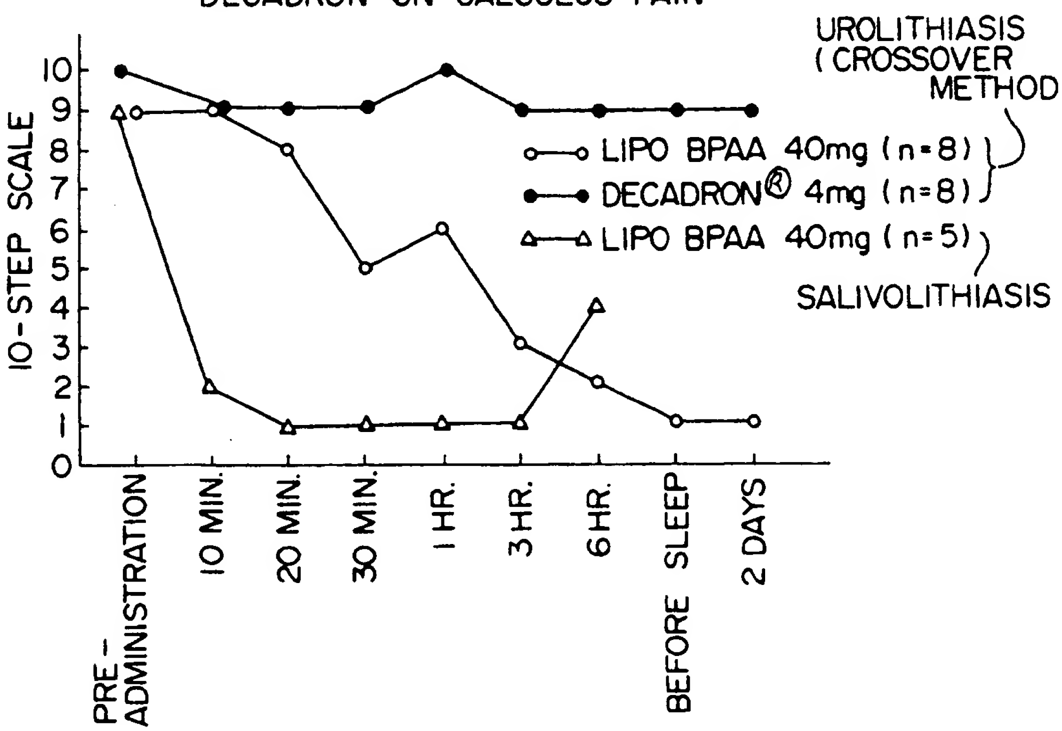


FIG. 5

COMPARISON OF ANALGESIC EFFCTS BETWEEN LIPO BPAA AND VENOPYRIN® BY CROSSOVER ADMINISTRATION IN R. A. PATIENTS (n=11)

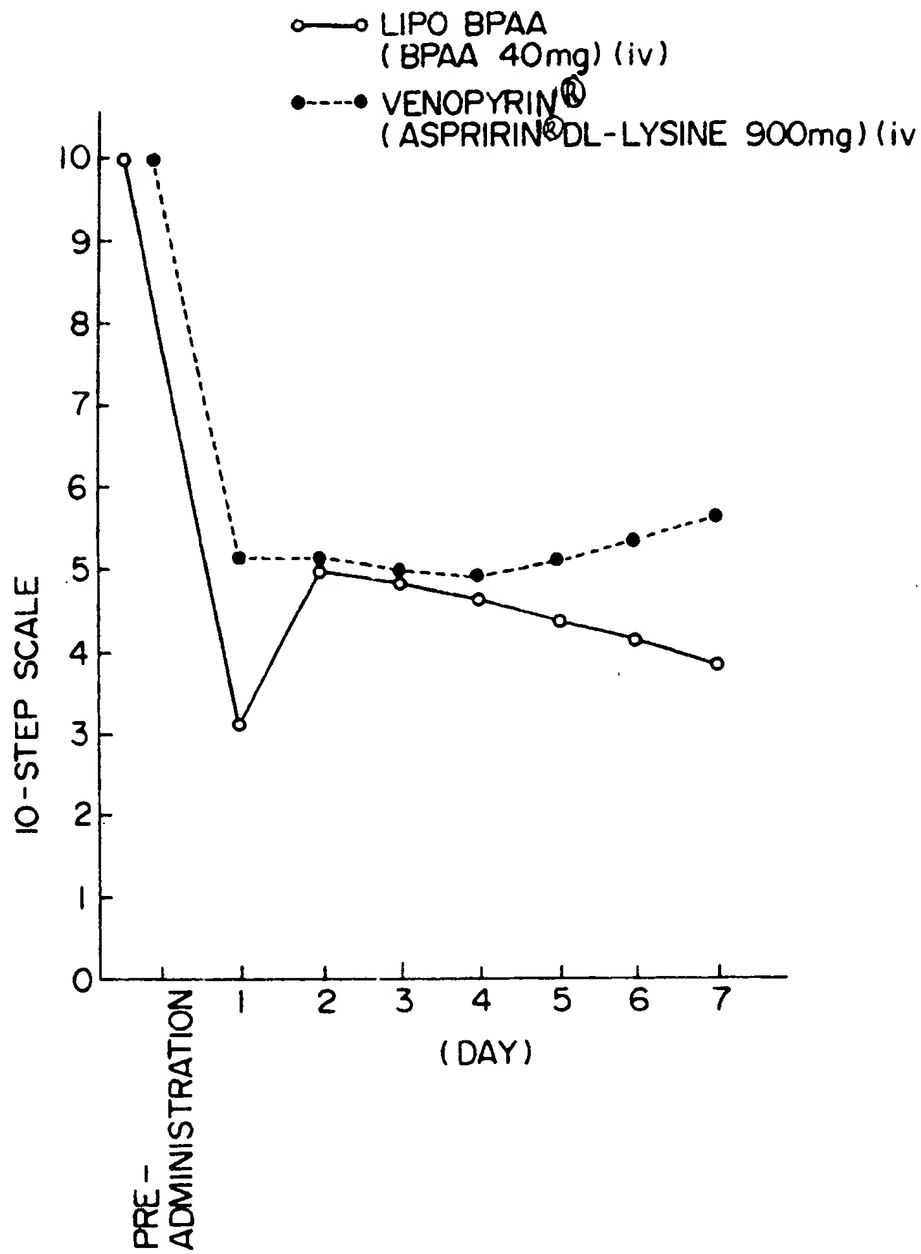


FIG. 6

IMPROVEMENT OF PHARYNGALGIA AFTER LIPO BPAA ADMINISTRATION IN PATIENTS WITH ACUTE UPPER RESPIRATORY TRACT INFLAMMATION (n=12)

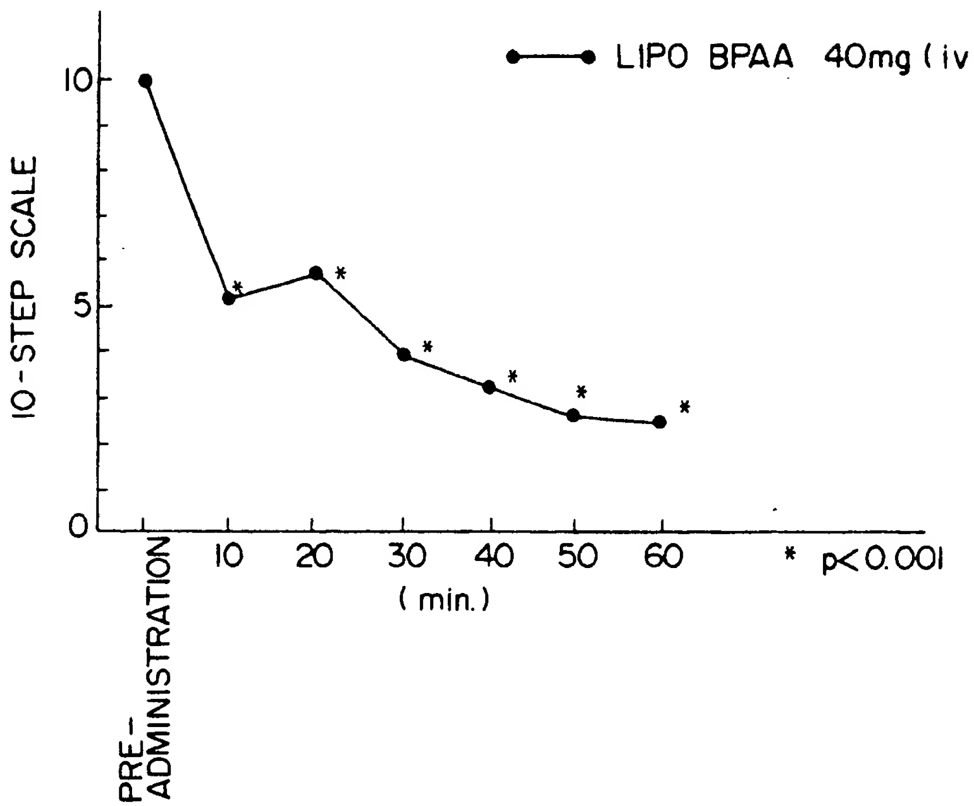


FIG. 7

ANALGESIC EFFCT OF LIPO BPAA ON CANCEROUS PAIN